

***Griffinia* (Amaryllidaceae), a Critically Endangered Brazilian Geophyte with Horticultural Potential**

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Abstract

The genus *Griffinia* consists of about a dozen species of rainforest or dry forest understory bulbous geophytes endemic to eastern Brazil. Two subgenera are recognized: subgenus *Griffinia*, found in coastal Atlantic Rainforest and its inland extensions, and subg. *Hyline*, an herb of sub-xeric *cerrado* or *caatinga* vegetation. The two species of subg. *Hyline* are nocturnal, white-flowered, and fragrant. The 10 species of subg. *Griffinia* are lilac to almost blue-flowered (rarely white). The species of subg. *Griffinia* are adapted to very low light levels, and have attractive petiolate leaves that are frequently spotted white. The species are characterized by $2n = 20$ chromosomes, but a preponderance of triploid individuals have been found in cultivation in Brazil. In addition to being exceedingly endangered by habitat destruction, the species have great unexploited horticultural potential as containerized plants and landscape perennials in tropical regions. Much basic work remains to be done on the genus; for example, nothing is known about its flowering physiology. One species, *G. hyacinthina*, has been successfully micropropagated in Brazil. Development of *Griffinia* as a floricultural crop must also be pursued with attention to the germplasm rights of the country of origin.

INTRODUCTION

Griffinia Ker Gawler is a small and critically endangered genus (Dean 1995; Walter and Gillet 1998) endemic to Brazil. The genus, established by Ker Gawler (1820), was named in honor of Mr. Griffin, a botanist/horticulturist of South Lambeth, England. The type species for both the genus (Ker Gawler 1820) and tribe Griffinieae (Ravenna 1974a), *Griffinia hyacinthina* (Ker Gawl.) Ker Gawl., was originally referred to the genus *Amaryllis* L. (Ker Gawler 1816). By the end of the 19th century, seven species of *Griffinia* had been described. The genus remained unaltered until the mid-20th Century. In 1960, *G. rochae* Morel, a small-flowered *Griffinia* from the state of Rio de Janeiro, became the first new species in the genus described in almost one hundred years.

Traub and Moldenke (1949) recognized six species. Since then, the monotypic genus *Hyline* Herbert, founded in 1841, has been reduced to a subgenus of *Griffinia* (Ravenna 1969a; *Griffinia* subg. *Hyline* Rav.), and a second species in that subgenus was described (Ravenna 1969b). Additionally, five species belonging to subg., *Griffinia* have been described (Morel 1960, 1961; Ravenna 1969c, 1974b, 1974c, 1978; Preuss and Meerow 2000). In all, twelve species are currently recognized (Preuss 1999), ten in subg. *Griffinia* and two in subg. *Hyline* (Table 1).

MATERIALS AND METHODS

Karyology

Root tips were harvested and treated with 0.05% colchicine at room temperature for six hours. Tips were rinsed three times with deionized water then were fixed in

Carnoy's solution (3 EtOH : 1 acetic acid) and refrigerated for 24 hours. The tips were then rinsed three times with and stored in 70% EtOH at 4° C. Tips were hydrolized in 1.0 N HCl at 54 °C for three minutes, rinsed three times in deionized water, and treated with 45% glacial acetic acid for 10 minutes. Slides were prepared by macerating a root tip and staining with 1.0% acetic orecein. The slides were observed on a Leitz Diaplan and cells undergoing mitotic metaphase were photographed at 1000x with a Wild MPS 51S using Kodak T-max 100 film.

Pollen Fertility

Pollen was collected from dehiscent anthers, either fresh, preserved in FAA or EtOH, or from herbarium specimens. Representatives from ten different collections were included (Table 3-2). Pollen was placed into a 1.5 ml microfuge tube with approximately 100 µl of the Alexander's (1969) stain. The sample was heated at 50 °C for 48 hours. Pollen grains were placed onto slides, coverslipped, and examined under the microscope. Pollen staining red was scored as viable and pollen staining light green was scored as nonviable. For each sample, 200 pollen grains were counted and scored. Percentage of viability was calculated.

Morphology

The bulbs of *Griffinia* are tunicated, and offset by production of daughter bulbs and/or bulbils from rhizomes, which appear root-like. The petiolate leaves range in form from elliptic to ovate, and are slightly falcate in subg. *Hyline* (a character state shared with *Worsleya*). The lamina, which has a distinct midrib, has pronounced parallel venation intersected by transverse striae, and may be variously speckled whitish in some species. The scape is solid, ancipitous (two-edged), and tumbling. The two spathe bracts are partially fused at the base and overlapping, persistent, and enclose a 2-20 flowered umbel of lilac and/or white zygomorphic flowers. The genus is unique in the Amaryllidaceae for the presence of a true hypanthium, formed by the continuation of the perigonal tube over the ovary in some of the species (Ravenna 1969; Preuss 1999). The positioning of the perigone is either epiperigynous (i.e. the floral tube surrounds the inferior ovary and forms a true hypanthium) or epigynous (i.e. the floral tube is inserted at the top of the ovary). The filiform style is declinate and the stigma is capitate. The stamens are arranged in either a 5 + 1 manner (*Griffinia* subg. *Griffinia*) or all 6 fascicled and declinate (*Griffinia* subg. *Hyline*). The sixth stamen is sometimes obsolete in certain species of subg. *Griffinia*, but this character is not consistent. The fruit is an angular, ovoid, tri-loculicidal, dehiscent capsule, typically with one seed. The seed lacks phytomelan, has a whitish testa, and is turgid and (sub-) globose. The chromosome number for the genus is $2n = 20$ (Fig. 1), although $2n = 30$ occurs in some individuals (Preuss 1999).

The morphology of the two subgenera is divergent, primarily in the flowers. In *Griffinia* subg. *Griffinia*, the leaves are symmetrical and vary from narrowly elliptic to broadly ovate. The lamina may be speckled whitish in some species (e.g. *G. liboniana* Morren and *G. espiritensis* Rav.). The inflorescence is 4–20-flowered. The flowers are variable in size, ranging from 1.5 cm. to about 11 cm long, diurnal, unscented, and lilac and/or white. The perigonal tube is reduced in the small, blue-flowered species. The tepals are arranged in a 5 + 1 pattern, giving a bilabiate appearance. In the small epigynous species, the lowermost tepal is narrow, revolute and divaricate. Five stamens are declinate, and variable in length (two long and three short), and the sixth (when present) is assurgent. The pollen is white. The number of ovules per locule ranges from 2-10. The chromosome number is $2n = 20$ for most species, however, for some forms of *G. liboniana* and *G. espiritensis* the chromosome number is $2n = 30$, presumably triploid in origin. Most of these triploid individuals show reduced pollen fertility (Preuss 1999).

In *Griffinia* subg. *Hyline* the leaves are slightly falcate and narrowly elliptic. The inflorescence is 2–3-flowered, and the flowers are large, nocturnal, scented, and white. The perigone is epiperigynous and fused into a tube, albeit highly variable in length. The

perianth is not as strongly zygomorphic as in subgenus *Griffinia*. All six stamens are fascicled, in three ranks of two, and declinate. The pollen is yellow. The number of ovules per locule ranges from 10-16. In both species of the subgenus, the chromosome number is $2n = 20$ (Mookerjea 1955; Preuss 1999).

Geographic Distribution

Complete distribution patterns for the species of *Griffinia* are not yet fully known, but appear to be disjunct. The plants are now rarely found in the remnants of the Brazilian Atlantic Forest, or Mata Atlântica, estimated to have been reduced to less than 8% of its former range over the past 500 years (Dean 1995). Populations of *Griffinia* are small, and occur as isolates in the remnants of the original vegetation of the Mata Atlântica (subg. *Griffinia*) and the dry cerrado and caatinga of the interior and the Northeast (subg. *Hyline*). Distribution patterns of both plants and animals in northeastern and eastern Brazil indicate the expansion of open vegetation formations (i.e. savannas, campos, cerrado, or caatingas) and accompanying forest contractions during the late Quaternary (Ledru 1993). The expansion and contraction of various forests throughout the Quaternary, characterized by “great environmental instability”, was responsible for speciation events for a large number of tropical taxa (Bigarella and de Andrade-Lima 1982) and is likely to have influenced the evolution of *Griffinia* as well.

The state of Rio de Janeiro has yielded collections for the type specimens of *G. hyacinthina*, *G. ornata* Moore, *G. intermedia* Lind., *G. rochae*, as well as the monotypic *Worsleya rayneri* (Hooker, f.) Traub and Moldenke, which DNA sequences indicate as the sister genus to *Griffinia* (Meerow et al. 2000a, b). This region may have been a center of diversity (or origin) for the tribe and its two genera. Though the type localities for *G. aracensis* Rav., *G. itambensis* Rav., and *G. liboniana* are from the state of Minas Gerais, material referable to at least *G. aracensis* and *G. liboniana* has been collected from the state of Bahia (Preuss 1999). *Griffinia parviflora* Ker Gawl. and *G. espiritensis* occur in Bahia and Espírito Santo to the south. Collections of *G. gardneriana* (Herb.) Rav. from Ceará and Maranhão, and *G. alba* Preuss and Meerow, from Pernambuco represent the northeastern limit of distribution for the genus. The distribution of *Griffinia* subg. *Hyline* (*G. gardneriana* (Herb.) Rav. and *G. nocturna* Rav.) is broad. Plants occur in seasonally dry regions, and collections have ranged from Mato Grosso do Sul, Tocantins, and Pará, to Bahia, Pernambuco, Maranhão, and Ceará.

Karyology

Griffinia demonstrates two chromosome numbers: $2n = 20$ and $2n = 30$ (Fig.1). Morel's (1960) report of $2n = 22$ for *G. rochae* was either in error or included B chromosomes in the complement. In both known species of subgenus *Hyline*, the chromosome number is $2n = 20$ (Mookerjea 1955; Preuss 1999). Thus $x = 10$ is presumed to be the basic chromosome number for the genus. Individuals of both the *Griffinia liboniana* complex and the *G. espiritensis* group demonstrate a derived putative triploid state, which could have arisen by fertilization of unreduced, diploid gametes either through apospory or pseudogamous embryo development, (Stebbins 1950). Alternatively, these $2n = 30$ individuals may be the result of hybridization between a diploid ($n = 10$) and a naturally occurring tetraploid ($n = 20$).

Four out of the five collections with $2n = 30$ were obtained from cultivated plants; only one was reportedly collected from its native habitat. The species and varieties with 30 chromosomes are more robust forms of the small, blue-flowered taxa. Perhaps horticulturists in Brazil had selected these individuals due to “gigas” (Stebbins 1950) effects of triploidy on vigor and plant size. Increased flower and plant size may be correlated with increased chromosome number in amaryllids (Bell 1973; Flory 1977). Any attempts to cross individuals of $2n = 20$ with $2n = 30$ have failed, while one out of five attempts to cross a $2n = 30$ individual with another $2n = 30$ individual from the same species complex resulted in the production of viable seed. At this time, the origin of the chromosome number of $2n = 30$ is uncertain.

Percentages of aborted pollen ranged from 100% in *G. liboniana* to 0.5% in *G. espiritensis* (Table 2). In general, plants with $2n = 20$ chromosomes had low percentages of aborted pollen, with the exception of one plant of *G. espiritensis*, while plants with the chromosome number of $2n = 30$ exhibited an increase in aborted pollen grains.

Phylogeny/Classification

Based upon phylogenetic analysis of sequence data generated from the internal transcribed spacer regions (ITS) of nuclear ribosomal DNA (Meerow et al. 2000a, b; Preuss 1999) and also morphological characters (Preuss 1999), a monophyletic tribe Griffinieae can be recognized. The tribe includes the monotypic sister genus *Worsleya*, and a monophyletic genus *Griffinia*. This lineage is suspected to be the oldest of the neotropical Amaryllidaceae (Meerow et al. 2000b).

A combined cladistic analysis of morphological characters and ITS sequences resolved the subgenera of *Griffinia* each as monophyletic (Preuss 1999), but only subg. *Griffinia* has bootstrap support (Fig. 2). Within subg. *Griffinia*, the epiperigynous, large-flowered species form a clade that is sister to the rest of the subgenus. The next branch encompasses the smaller-flowered, epiperigynous species. The small-flowered, epigynous species are a well-supported clade (the *Griffinia liboniana* complex). Within this informally recognized group, there are several species whose inter- and infraspecific relationships are complex and uncertain. *Griffinia liboniana* is the oldest named taxon. The group is characterized by petiolate to subpetiolate leaves, solid green or variously speckled white; petiole and scape lacking reddish pigmentation; epigynous insertion of floral parts; the perigonal tube reduced and not continuous with the pericarp; and the upper episepal stamen typically lacking, or, when present, not adpressed to the dorsal tepal.

Ecological Pressures

Most species of *Griffinia* grow in the lush, wet understory of tropical forests of eastern Brazil. The Brazilian Atlantic Forest has undergone tremendous modification (Dean 1995). The once contiguous forests of the Mata Atlântica, which extended inland from the coast about 100 kilometers in the north and more than 500 kilometers in the south, encompassed about a million square kilometers and ranged from 8° to 28° South latitude (Dean 1995). A survey of the entire Atlantic Forest showed that by 1990 only a little more than eight percent, or 83,500 square kilometers, remained (Farez 1993). Contiguous chunks of forest are now rare. Like *Eucharis* Planch. & Lind. (Meerow 1989), the blue-flowered *Griffinia* are primary rain forest elements, and do not re-colonize readily where forest disturbance has been manifold.

Upon visiting several previous collection sites of *Griffinia* in the state of Bahia, it was evident that most sites were no longer able to sustain once extant populations due to extreme modification of the terrain. Like many other genera of tropical plants and animals, *Griffinia* is experiencing ecological pressure from the modification and loss of its native tropical forests.

Horticultural Perspective

Members of *Griffinia* subg. *Griffinia* are cultivated in heirloom gardens throughout their range, where they are referred to as *carícia*. When inquiries are made about the origins of cultivated plants, the answer is usually that they were collected from native forests in the vicinity before they were cleared. The *Hyline* group are of interest only to collectors, and have never been observed in cultivation in Brazil outside of botanical gardens. Griffiniads have much to offer horticulture. They are adapted to low light levels and are at least semi-evergreen. *Griffinia liboniana* in particular has ornamental foliage. The lilac-blue color of flowers is the main ornamental feature of the species. *Griffinia hyacinthina* has the largest flowers in subg. *Griffinia*, and is currently a focus of a micropropagation effort in Brazil.

Little is known about the flowering physiology or periodicity of the genus, and our

observations are anecdotal. *Griffinia liboniana* and *G. espiritensis* are the easiest to maintain and flower without much effort. Triploid clones of *Griffinia espiritensis* in particular appear to be more free-flowering than other species.

In cultivation in Florida, most of the species demonstrate a short period of dormancy with leaf senescence. It is unclear if this is a response peculiar to our conditions or has correspondence with cycles experienced in habitat. Selections are being propagated for greenhouse and growth chamber studies on the effects of temperature and photoperiod on flowering.

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Tables

Table 1. Described species and synonyms for the genus *Griffinia* (including *Hyline*). An asterisk indicates species recognized by Preuss (1999).

* <i>Griffinia hyacinthina</i> (Ker Gawl.) Ker Gawl., <i>Edwards Bot. Reg.</i> 6. Sub t. 444.;
<i>Amaryllis hyacinthina</i> Ker Gawl., <i>Edward's Bot. Reg.</i> 2: sub t. 163. 1816;
<i>Lycoris hyacinthina</i> Herbert, <i>Curtis' Bot. Mag.</i> 47: sub t. 2113. 1819.
* <i>Griffinia alba</i> Preuss & Meerow, Novon
* <i>Griffinia parviflora</i> Ker Gawl., <i>Edward's Bot. Reg.</i> 6: sub t. 511. 1821.
* <i>Griffinia intermedia</i> Lindley, <i>Edwards Bot. Reg.</i> 7: sub t. 990. 1826
<i>Griffinia dryades</i> (Vellozo) Vellozo, <i>Flor. Flum. Index</i> , 3.;
<i>Amaryllis dryades</i> Vellozo, <i>Flor. Flum. Liber primus</i> . 130, Icones, vol. iii. Sub t.
* <i>Griffinia liboniana</i> Morren, <i>Ann. Soc. Roy. Agr. Bot.</i> 1: 143. 1845;
<i>Liboniana bicolor</i> Lemaire in <i>Jard. Fleur.</i> vol. iii: sub t. 290. 1853.
* <i>Griffinia ornata</i> Moore, <i>Gard. Chron.</i> I: 266. 1876;
* <i>Griffinia rochae</i> Morel, <i>Baileya</i> , vol. 8: 133. 1961; and in <i>Baileya</i> , vol. 9: 28. 1961.
* <i>Griffinia espiritensis</i> Ravenna, <i>Plant Life</i> , 25: 67. 1969.
* <i>Griffinia gardneriana</i> (Herbert) Ravenna, <i>Plant Life</i> , 25: 62-63. 1969.
<i>Hyline gardneriana</i> Herbert, <i>Curtis's Bot. Mag.</i> 66: sub t. 3779. 1841.
<i>Hyline Worsleyi</i> Mallet, <i>Gard. Chron.</i> III: 26. 102. 1899.
* <i>Griffinia nocturna</i> Ravenna, <i>Plant Life</i> , 25: 62-63. 1969.
* <i>Griffinia aracensis</i> Ravenna, <i>Plant Life</i> , 30: 69. 1974; and in <i>Plant Life</i> 34: 82. 1978.
* <i>Griffinia itambensis</i> Ravenna, <i>Plant Life</i> , 30: 70. 1974.
<i>Griffinia rostrata</i> Ravenna, <i>Plant Life</i> , 34: 82. 1978.
<i>Griffinia concinna</i> (Martius) Ravenna, <i>Plant Life</i> , 27: 84. 1971.
<i>Crinum concinnum</i> Mart., in Roemer et Schultes, <i>Syst. Veg.</i> , 7: 857. 1830.
¹ <i>Griffinia blumenavia</i> Koch & Bouche; in Carriere, <i>Rev. Hort.</i> 39: 32-33. 1867.
<i>Hippeastrum blumenavium</i> (Koch & Bouche, ex Carr.) Sealy, <i>Curtis' Bot. Mag.</i> 160: sub t. 9504. 1937.
¹ <i>Griffinia mollevillquensis</i> (Cárdenas) Traub, <i>Plant Life</i> , 39: 16. 1983.
<i>Amaryllis mollevillquensis</i> Cárdenas, <i>Plant Life</i> , : 29-31. 1962.
<i>Hippeastrum mollevillquense</i> (Cárdenas) Van Scheepen, <i>Taxon</i> , 46: 15-19. 1997.
¹ <i>Griffinia incachacana</i> (Cárdenas) Traub, <i>Plant Life</i> , 39: 16. 1983.
<i>Amaryllis incachacana</i> Cárdenas, <i>Plant Life</i> , 21: 54-55. 1965.
<i>Hippeastrum incachacanum</i> (Cárdenas) Van Scheepen, <i>Taxon</i> , 46: 15-19. 1997.
¹ These taxa are excluded from the genus <i>Griffinia</i> and are recognized under the genus <i>Hippeastrum</i> .

Table 2. Results of pollen viability test of selected *Griffinia* populations using Alexander (1969) stain.

Accession #	Species	Chromosome #	% Viable Pollen	Source (Cultivation or wild)
95-4B	<i>G. parviflora</i>	$2n = 20$	97.5 %	Wild
97G-2	<i>G. aracensis</i>	$2n = 20$	82.6 %	Cultivation
95-79A	<i>G. espiritensis</i>	$2n = 30$	67%	Reportedly wild
97G-4B	<i>G. espiritensis</i>	$2n = 20$	99.5 %	Cultivation
97-G5	<i>G. espiritensis</i>	$2n = 30$	54%	Cultivation
97-G3A	<i>G. espiritensis</i>	$2n = 20$	41%	Cultivation
97-G7	<i>G. espiritensis</i>	$2n = 20$	68%	Wild
95-22A	<i>G. espiritensis</i>	$2n = 30$	93 %	?
s. n.	<i>G. liboniana</i>	$2n = 30$	34 %	?
95-3B	<i>G. liboniana</i>	$2n = 30$	0%	Cultivation

Figures

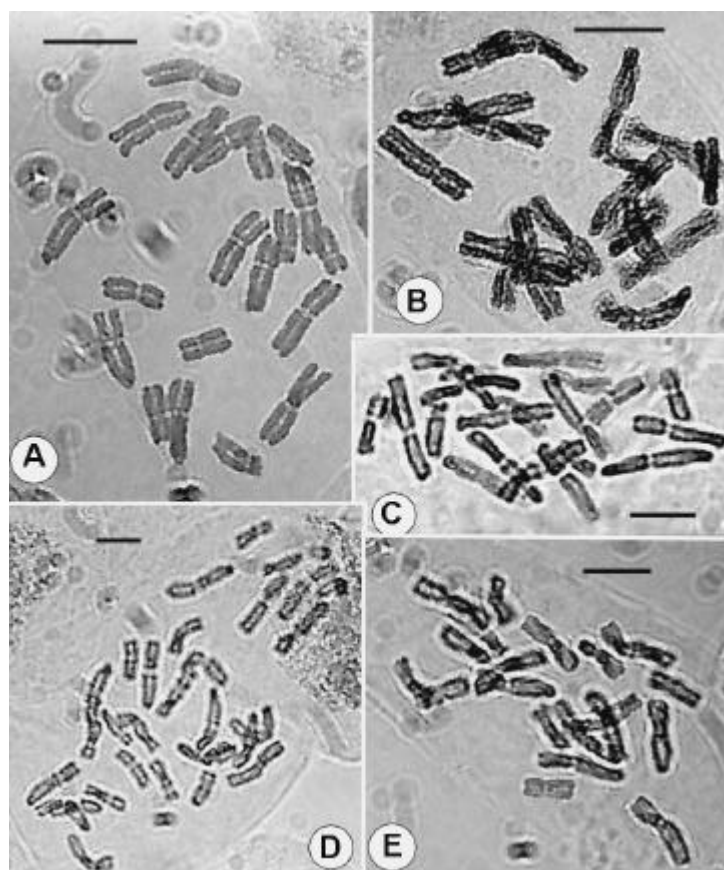


Fig. 1. Chromosomes of *Griffinia*. A. *G. aracensis*, $2n = 20$. B. *G. espiritensis*, $2n = 20$. C. *G. liboniana*, $2n = 20$. D. *G. liboniana*, $2n = 30$. E. *G. hyacinthina*, $2n = 20$. Scale line = 10 μ m.

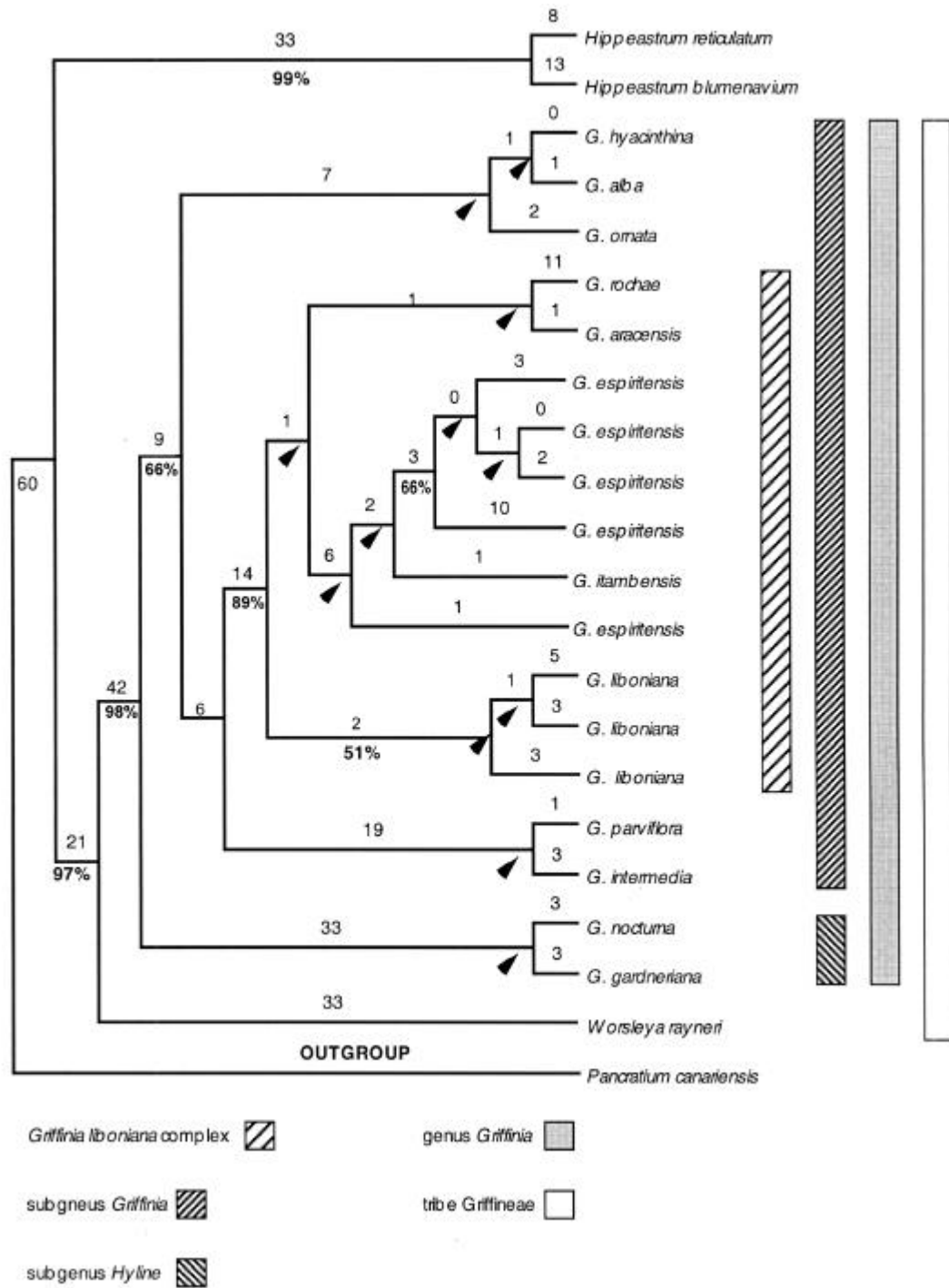


Fig. 2. Selected tree from cladistic analysis of combined nuclear RNA ITS sequences and morphology matrices for *Griffinia* (Preuss 1999). Arrows indicated branches that are not supported in the strict consensus of all trees. Numbers above branches are branch lengths. Percentages below branches represent bootstrap support.